Homozygous RRM2 Variant Might Lead to Early Embryo Developmental Arrest: A Case Report

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Case Report

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Abstract

Background: Early embryonic developmental stagnation is one of the reasons that affect the outcome of *in vitro* fertilization-embryo transfer, leading to the depletion of available embryos or failure after transplantation. It has been shown that defects in the ribonucleotide-reductase lead to cell cycle arrest, developmental delay, and high mutation rates.

Case presentation: Two female patients, who were siblings from an inbreeding family, suffered primary infertility of unknown causes and early embryonic developmental arrest during IVF treatment. A total of 39 oocytes were obtained from the patients in collectively 5 IVF/ICSI cycles, of which 37 were mature eggs, only 2 transplantable embryos were formed, and no pregnancy was achieved. Whole genome sequencing and Sanger sequencing were adopted to identify and confirm variations that might cause early embryo developmental stagnation in this family. We identified a homozygous variant c.262C>T:p.His88Tyr in ribonucleotide-diphosphate reductase subunit M2 (RRM2) in both patients and their parents each carried a heterozygous allele. Pedigree analysis showed an autosomal recessive inheritance pattern. Function of this variant was predicted by online databases, which indicated it to be a potential pathogenic mutation.

Conclusions: We identified *RRM2* as a potential causative gene for early embryonic developmental stagnation. It was also suggested that *RRM2* might be a maternal effect gene.

Background

Infertility is one serious issue that threatens the human reproductive health. According to statistics, on a worldwide scale 15% of married couples are infertile, and infertility factors affect about half of men and women (1). The development of assisted reproductive technology brings hope to many infertile patients. Studies have shown that 40%-70% of human embryos in *in vitro* fertilization (IVF) cycles are living embryos, whereas the others are arrested at different developmental stages (2). Stagnant embryos are usually discarded, and live embryos are frozen, continue to be cultured into blastocysts, or directly transplanted back into the patient's uterus. However, in some cases the development of all embryos from a patient are arrested at early developmental stages, leading to failed IVF attempt, which cannot be rescued by multiple embryo transplantation or single sperm microinjection. The cause of such condition is speculated to relate to genetic factors.

At present, research on the genetic factors of human embryonic stagnation is very limited. Up to now, there are only two reported gene mutations that could cause human embryo development stagnation and infertility. A homozygous variation in *TLE6* gene was first reported to account for the stagnation of early embryonic development in human, which was identified by exome sequencing in an Arab inbreeding family (3). *TLE6* is a maternal effect gene. In the early stage of human embryo development, especially from fertilization to the 8-cell stage, gene transcription is not fully initiated. The basic biological processes of cells including protein synthesis and regulation of embryo development depend almost entirely on the RNA stored in the egg. Therefore, the mutation of maternal effect genes may affect the development and survival of fertilized eggs and embryos. In another study, homozygous mutations in *PADI6* gene in a Chinese inbreeding family resulted in the stagnation of fertilized eggs and embryos from the female patient (4). In the meantime, compound heterozygous mutations of *PADI6* gene were found in 2 of 36 sporadic cases of early embryonic development arrest (4). Interestingly, *PADI6* is also a maternal effect gene, which again indicated the important role of maternal factors in early embryonic development.

In this study, we reported the identification of a homozygous variation NM_001034: exon3: c.262C > T: p.His88Tyr in ribonucleotide-diphosphate reductase subunit M2 (*RRM2*) in two infertile sisters from an inbreed family, who both suffered primary infertility and early embryo developmental arrest. RRM2 was also identified as a potential maternal effect factor.

Case Presentation

Patients

Two female patients P1 and P2 (Table 1), who were siblings from an inbreeding family (Fig. 1A), were diagnosed of primary infertility. Both patients were in good physical condition, and had regular sexual intercourse of 2–3 times a week. Through physical examination, ultrasonography, hystero-salpingography and laparoscopic exploration, we found no abnormalities in the patients' vulva, vagina, cervix, ovary, fallopian tubes, and uterus. Periodic development of follicles was monitored by transvaginal ultrasound. Blood gonadal hormone was detected on the second day after menstruation, and no abnormalities were observed in all indicators in either patient. The patients and their husbands all had normal karyotype. The reproductive system of both patients' husband was well developed, and no abnormalities were found in semen analysis. The two couples had sought medical advice elsewhere but failed to identify any potential cause of infertility and had been treated with empirical Chinese herbal medicine. Both patients received *in vitro* fertilization and embryo transfer (IVF-ET) in our institution (Table 2).

Table 1
Basic information of the two patients.

	P1	P2
Age (yrs)	38	35
Length of infertility (yrs)	11	7
Menstruation cycle (days)	30	35
Menstrual period (days)	5	7
Age of menarche (yrs)	14	17
BMI	25.4	25.6

Table 2
Summary of the patients' assisted reproduction treatment outcome.

Patient	Cycle	Fertilization method	Total oocytes obtained (N)	Immature oocytes (N)	Mature oocytes (N)	Unfertilized (N)	2PN	1PN	Multi prokaryotic (N)	Cleavage (N)	Transplantable embryos (N)
P1	1	IVF	5	0	5	1	4	0	0	3	2
	2	IVF	5	0	5	2	1	2	0	0	0
	3	ICSI	7	1	6	2	4	0	0	0	0
	4	IVF	6	0	6	1	3	0	2	0	0
P2	5	IVF	16	1	15	5	6	2	2	0	0
Total			39	2	37	11	18	4	4	3	2

A total of 39 oocytes were obtained from the patients in collectively 5 IVF/ICSI cycles, of which 37 were mature eggs. Two embryos were formed during three IVF cycles plus one ICSI cycle of P1, both of which failed to develop after transplantation. P2 went through one IVF cycle, in which no transplantable embryo was formed, and gave up treatment. The outstanding problem was that the fertilization rate of mature eggs (18/37, 48.6%) and the cleavage rate of 2PN cells (3/18, 16.7%) were both low.

Exome sequencing identified homozygous RRM2 variation

We performed whole exome sequencing as previously described (3) on the peripheral blood sample of P1 and focused on the identification of homozygous variations. The average depth of whole exome sequencing was greater than 100×. Through screening the gene polymorphisms with allele frequencies greater than 1% in the dbSNP, 1000 Genome and ESP6500 databases, we found a list of genes containing homozygous variations (Table 3), among which only *RRM2* is closely related to embryonic development potential. The homozygous variant in *RRM2* (NM_001034:exon3:c.262C > T:p.His88Tyr) was confirmed by Sanger sequencing as previously described (5) in both patients (Fig. 1B, 2A), and their parents each carried a heterozygous allele. Pedigree analysis showed an autosomal recessive inheritance pattern (Fig. 1A).

Table 3
List of homozygous gene variations identified by whole exome sequencing.

Gene	Chromosome	Variation	Exonic function
IGFN1	chr1	NM_001164586:exon12:c. 6119C > T:p.Ala2040Val	nonsynonymous SNV
OR8G2	chr11	NM_001291438:exon1:c. 850G > A:p.Gly284Arg	nonsynonymous SNV
DCHS1	chr11	NM_003737:exon2:c.99_100insCTG:p.Gly34delinsLeuGly	nonframeshift insertion
DACH1	chr13	NM_004392:exon1:c.244_249del:p. 82_83del, NM_080759:exon1:c.244_249del:p. 82_83del, NM_080760:exon1:c.244_249del:p. 82_83del	nonframeshift deletion
RIN3	chr14	NM_024832:exon10:c.2899_2901del:p. 967_967del	nonframeshift deletion
MKL2	chr16	NM_001308142:exon9:c.761C > G:p.Pro254Arg, NM_014048:exon9: c.761C > G:p.Pro254Arg,	nonsynonymous SNV
KIAA0430	chr16	NM_001184998:exon21:c.4181_4182insTGTCGTGAA:p.Lys1394delinsAsnValValLys, NM_001184999:exon21:c.4172_4173insTGTCGTGAA:p.Lys1391delinsAsnValValLys, NM_014647:exon21:c.4181_4182insTGTCGTGAA:p.Lys1394delinsAsnValValLys	nonframeshift insertion
COQ7	chr16	NM_016138:exon1:c.50C > T:p.Pro17Leu	nonsynonymous SNV
PKD1L2	chr16	NM_001076780:exon4:c.706_707del:p.Asn236fs	frameshift deletion
GSDMA	chr17	NM_178171:exon3:c.382G > T:p.Val128Leu	nonsynonymous SNV
PLIN4	chr19	NM_001080400:exon3:c.2580A > T:p.Lys860Asn	nonsynonymous SNV
RRM2	chr2	NM_001034:exon3:c.262C > T:p.His88Tyr, NM_001165931:exon3:c.442C > T:p.His148Tyr	nonsynonymous SNV
NCL	chr2	NM_005381:exon4:c.774_776del:p. 258_259del	nonframeshift deletion
MAP3K1	chr5	NM_005921:exon14:c.2822_2824del:p. 941_942del	nonframeshift deletion
MUC3A	chr7	NM_005960:exon11:c.4384C > T:p.His1462Tyr	nonsynonymous SNV
MEOX2	chr7	NM_005924:exon1:c.228_230del:p. 76_77del	nonframeshift deletion

In silico analysis of RRM2:c.262C > T:p.His88Tyr variant

In silico analysis predicted that RRM2:c.262C > T was a disease-associated mutation in human (Table 4). The allele frequency of RRM2:c.262C > T in the world population was very low, but was 10-fold higher in the East Asian population. However, homozygotes of this variant has not been documented in either 1000 Genomes or Exome Aggregation Consortium (ExAC) database. The c.262C > T point mutation in *RRM2* resulted in a substitution of histidine by tyrosine at the 88 amino acid. As histidine is a hydrophilic, positively-charged basic amino acid while tyrosine is a hydrophobic, neutral amino acid with aromatic residue, this substitution might affect the three-dimensional protein conformation, attenuating the stability and function of RRM2 protein (Fig. 2B). This histidine of RRM2 is highly conserved among different species (Fig. 2C), indicating its functional importance. The heterozygous variant of RRM2:c.262C > T carried by the patients' parents suggested that heterozygote of this variant is not pathogenic.

Table 4
In silico analysis of the RRM2 variant.

Mutation	Amino acid change	1000G_ALL[a]	1000G_EA[b]	ExAC (total)[c]	ExAC_EA[d]	ESP6500 siv2_ALL[e]	ESP6500 siv2_EA[f]	Polyphen-2[g]	SIFT[h]	Mutation Taster[i]	SNP
c.262C > T	p.His88Tyr	0.000599042	0.003	0.00004969	0.0005	NA	NA	Benign (0.025)	Tolerated (0.053)	Disease causing (1)	Dise (0.84

[a] Frequency of variation in total of 1000 Genomes database (A Deep Catalog of Human Genetic Variation).

[b] Frequency of variation in East Asian population of 1000 Genomes database.

[c] Frequency of variation in total of ExAC database.

[d] Frequency of variation in East Asian population of ExAC database.

[e] Frequency of variation in total of ESP6500 database.

[f] Frequency of variation in East Asian population of ESP6500 database.

[g] Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/). Prediction Scores range from 0 to 1 with high scores indicating probably or possibly damaging.

[h] SIFT, that is, Sorting Intolerant From Tolerant (http://sift.jcvi.org/). Scores vary between 0 and 1. Variants with scores close or equal to 0 are predicted to be damaging.

[i] Mutation Taster (http://www.mutationtaster.org/). The probability value is the probability of the prediction, that is, a value close to 1 indicates a high"securit the prediction.

[j] SNPs & GO (http://snps.biofold.org/snps-and-go/). Probability: disease probability (if > 0.5 mutation is predicted disease).

Discussion And Conclusions

This study identified the homozygous variant RRM2:c.262C > T:p.His88Tyr in two infertile patients that might be the potential cause of early embryonic development stagnation in their IVF-ET treatment, which resulted in depletion of all available embryos for transplantation. Bioinformatics analysis predicted this variant might affect RRM2 protein function and cause disease. According to the American College of Medical Genetics (ACMG) guidelines for classification of variants (6), this was classified as a "variant of uncertain significance".

Ribonucleotide reductase (RR) consists of two subunits (RRM1 and RRM2), and is a rate-limiting enzyme in deoxynucleotide production for DNA synthesis (7). As optimal cell concentration of deoxyribonucleotides is essential for DNA synthesis, replication, and repair (8), RR plays an important role in cell proliferation. RR defects often lead to cell cycle arrest, developmental delay, and abnormally increased RR activities led to high frequency of gene mutation (9). Functionally, RRM1 controls substrate specificity, and RRM2 regulates the overall catalytic activity (10). RRM2 is widely expressed in human organs, with highest expression level in secondary oocyte (data from Bgee data Base for Gene Expression Evolution).

RRM2 could regulate zygotic genome activation (ZGA) through the yes-associated protein (YAP). In early mammalian embryos, genomic transcription is quiescent until ZGA occurs 2–3 days after fertilization. The coding gene of YAP, *Yap1*, was found to be highly expressed in human and mouse oocytes and early embryos (11, 12), and maternally accumulated YAP in oocyte is essential for ZGA (1). The embryos of *Yap1* knockout female mice presented prolonged 2-cell stage and slower development into the 4-cell stage. *Rrm2*, the mouse homologous gene of human *RRM2*, and Rpl13 were found to be the target genes of YAP in early blastomeres, which were required to mediate maternal YAP's effect in conferring developmental competence on preimplantation embryos (13). This was in accordance with our observation that the embryonic development stagnation caused by *RRM2* variation exhibited maternal effect recessive inheritance. That is, female homozygotes for the *RRM2* mutation appeared phenotypically normal, whereas their offspring shown the mutant phenotype of lethality during early embryonic development.

RRM2 has also been reported to play an important role in inducing cell proliferation and decidualization in mouse uterus, suggesting its likely involvement in early embryo development and embryo implantation. Embryo implantation into the maternal uterus is a crucial step in the successful pregnancy of mammals. Currently, implantation and trophoblastic infiltration defects are major obstacles to successful pregnancy (14). Embryo implantation is a complex developmental process, including the process of embryo attachment, followed by invasion of the matrix, and proliferation and differentiation of endometrial stromal cells (15). It has been shown that Rrm2 is strongly expressed in decidual tissues and is up-regulated by progesterone and DNA damage in mouse (7). RRM2-specific inhibitors effectively reduced the weight of implantation sites and deciduoma (7). In the future, functional study by generating genetically modified mouse in the *Rrm*2 gene would help supporting the pathogenicity of this variant.

In summary, this study identified a homozygous variation of *RRM2*:exon3:c.262C > T:p.His88Tyr, which might alter RRM2 protein conformation and attenuate its function, and might be a potential cause of early embryonic development stagnation in two sisters who had multiple failed assisted reproduction attempts. This study also suggested that *RRM2* might be a maternal effect gene. Further animal study is still required to confirm the role of RRM2 in early embryo development.

List Of Abbreviations

RRM2, ribonucleotide-diphosphate reductase subunit M2; IVF-ET, *in vitro* fertilization and embryo transfer; ExAC, Exome Aggregation Consortium; ZGA, zygotic genome activation; YAP, yes-associated protein

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Yantai Yuhuangding Hospital for use of human sample. Informed consent was obtained from all participants for enrolling in the study prior to their inclusion in the study.

Consent for publication

Informed consent was obtained from all participants for the use of personal information for publication.

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due to privacy and ethical restrictions but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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No funding was received.

Authors' contributions

XW, CFH, and JFW conceived of this study. XW, ZTL and XYL performed medical examinations and IVF-ET treatment and collected information. YWS, HLW and JFW carried out gene sequencing and genetic analysis. XW and JW analyzed data and wrote the manuscript.

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Figures

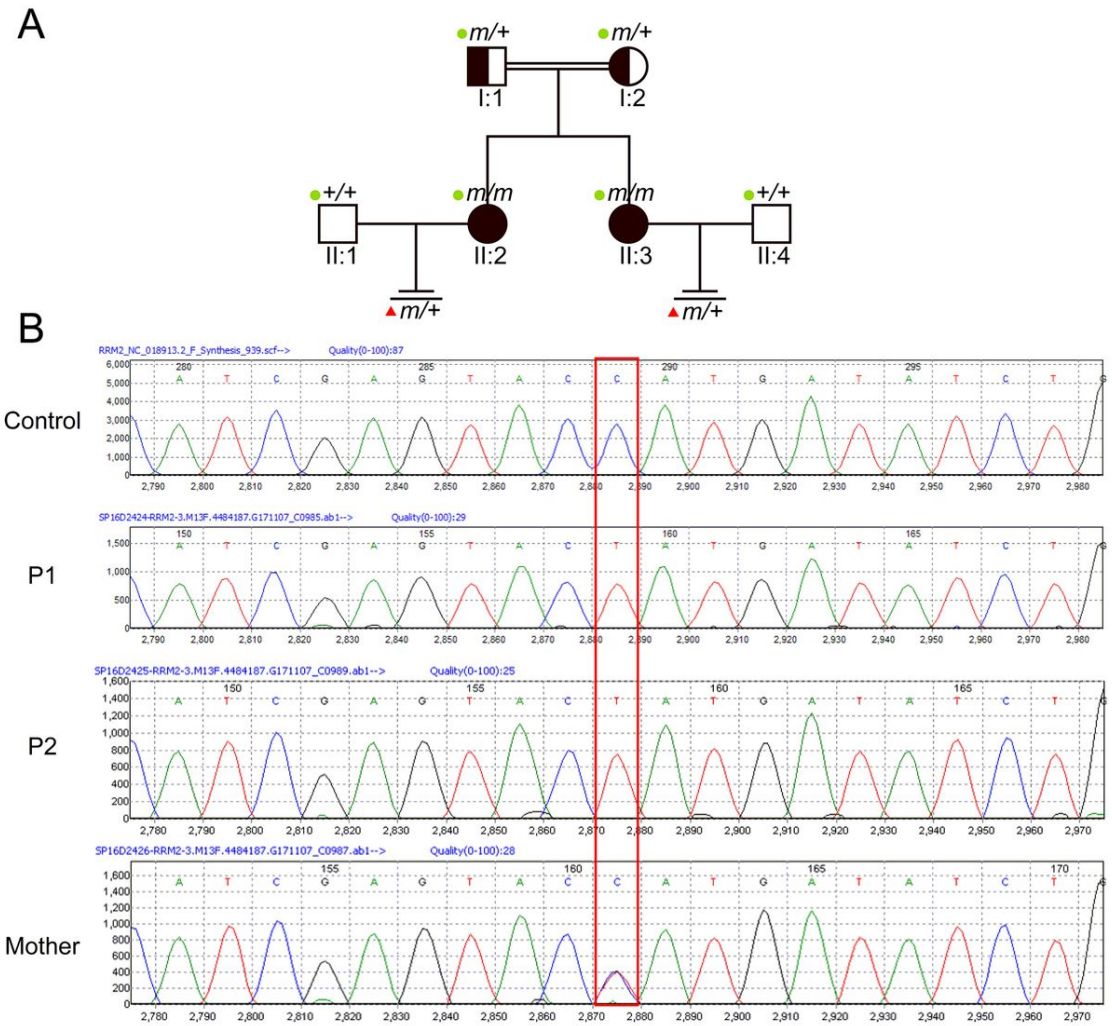

Figure 1

(A) Pedigree of the patients' family. m: mutated allele of RRM2; +: normal allele; green dots: normal phenotype; red triangle: embryonic development failure phenotype.

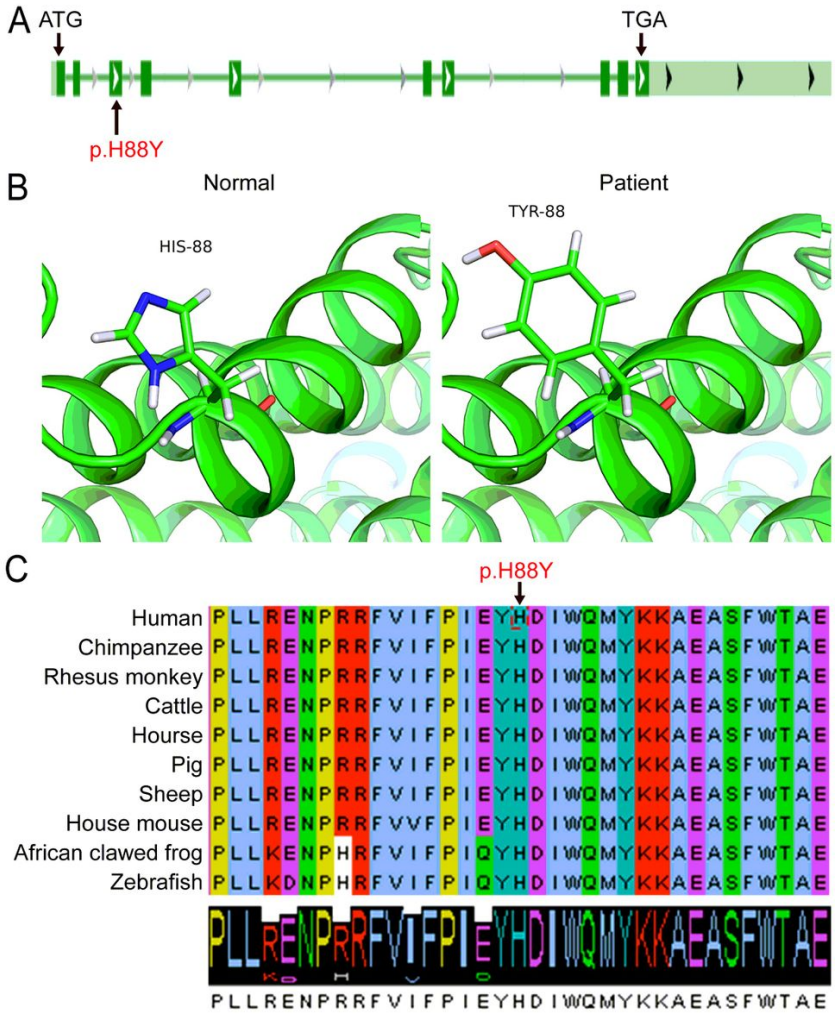

Figure 2

(A) Diagram of the RRM2 protein. (B) Diagram showing the substitution of the histidine by tyrosine in RRM2 protein of the patients. (C) Homology analysis of RRM2 protein among difference species.